

Grazing and food selection of the calanoid copepods *Eurytemora affinis* and *Acartia bifilosa* feeding on plankton assemblages containing *Dinophysis* spp.

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Grazing of *Eurytemora affinis* and *Acartia bifilosa* on plankton communities containing toxic dinoflagellates of the genus *Dinophysis* was experimentally studied in the northern Baltic Sea. The experiments were carried out in plankton communities with different concentrations and relative proportions of microplankton. In the experiments with high total cell concentrations, the dinoflagellate *Heterocapsa triquetra* predominated, and in these experiments they formed the main part of the copepod diet. When the total cell concentrations were lower and the relative *Dinophysis* spp. concentrations higher, the role of *Dinophysis* spp. as food became more important, although no marked change in the ingestion rates were found. *Eurytemora affinis* consumed up to 226 *Dinophysis* spp. cells (0.50 µg C) ind⁻¹ day⁻¹, and *A. bifilosa* up to 64 cells (0.1 µg C) ind⁻¹ day⁻¹. No positive selection for *Dinophysis* spp. was detected, and in fact, other species were preferred. Pectenotoxin-2 (PTX2) was found in two pooled samples of copepods after incubation (53 and 142 pg PTX2 ind⁻¹, respectively). Although no positive selection for *Dinophysis* spp. was detected, these dinoflagellates formed an important part of the copepod's diet at low food concentrations in these experiments. It is thus possible that in environmental conditions characterized by low microplankton biomass, the copepods can have a minor role in toxin transfer in planktonic food webs of the northern Baltic Sea by accumulating toxins in their tissues or transferring them to faecal pellets.

Introduction

Dinoflagellates of the genus *Dinophysis* produce toxic compounds commonly known as diarrhetic shellfish poisoning (DSP) toxins. The DSP toxins frequently contaminate filter-feeding shellfish in marine coastal areas causing great

economic losses and public health problems. In the northern Baltic Sea such problems do not exist, since the brackish water mussels are too small for commercial use. Three toxic *Dinophysis* species — *Dinophysis acuminata*, *D. norvegica* and *D. rotundata* — occur commonly in the northern Baltic Sea. The *Dinophysis* spp.

abundances in the area vary typically between 1 and 5 cells ml⁻¹, but also layers of higher concentrations of up to 150 cells ml⁻¹ have been found (Carpenter *et al.* 1995). In the northern Baltic Sea DSP toxins were first detected in the Gulf of Finland where okadaic acid (OA) in blue mussels (Pimiä *et al.* 1998) and in the common flounder (Sipiä *et al.* 2000). Studies in the northern Baltic have found other *Dinophysis*-derived toxins such as pectenotoxins (PTX2), and dinophysistoxins (DTX1 and DTX3) in phytoplankton samples containing *Dinophysis* spp. (Goto *et al.* 2000, Kozłowsky-Suzuki *et al.* 2006, Kuuppo *et al.* 2006). Okadaic acid has not been found in phytoplankton communities, despite the findings of OA in benthic animals.

The factors that control the development of harmful algal blooms are complex, and include e.g. hydrodynamic and meteorological events (Godhe *et al.* 2002), variations in local inorganic and organic nutrient concentrations and balances (Anderson *et al.* 2002), and interactions with other organisms. Biological factors, such as allelopathy (Guisande *et al.* 2002), parasites (Coats *et al.* 1996) and grazing (Turner and Tester 1997, Turner *et al.* 1998) can affect the fate of algal populations. Since zooplankton grazing is an important factor affecting phytoplankton growth, it is essential to study the relationships between toxic algal species and zooplankton, and the role of zooplankton in toxin transfer. The transport of marine biotoxins through food webs may be an important route to toxin accumulation at higher trophic levels. Algal toxins that are transported via the grazing food chain may affect marine animals such as fishes (White 1981, Beaulieu *et al.* 1996, Castonguay *et al.* 1997) or birds (Sierra-Beltran *et al.* 1997, Shumway *et al.* 2003) that do not directly feed on toxin-producing phytoplankton.

The effects of toxic algae on grazing zooplankton are diverse and vary among algae and zooplankton species and seem to be highly species- and site-specific. Algal toxicity is often seen as sublethal effects in copepod grazers. Such effects include changes in behaviour and food intake and reduction in fecundity (*see* Turner *et al.* 1998 and references therein). Copepods have, however, the ability to feed on various toxic phytoplankton species, including species that pro-

duce paralytic shellfish poisoning (PSP) toxins and DSP toxins (Carlsson *et al.* 1995, Bagøien *et al.* 1996, Colin and Dam 2003, Kozłowsky-Suzuki *et al.* 2006). Algal toxins may be accumulated in zooplankton tissues (Turner *et al.* 2000), eggs (Frangopulos *et al.* 2000) as well as faecal pellets (Maneiro *et al.* 2000, Maneiro *et al.* 2002, Kuuppo *et al.* 2006).

The objectives in this study were to test (1) whether the calanoid copepods in the area feed actively on *Dinophysis* species, and thus (2) could they potentially act as vectors for toxin transport to higher trophic levels in planktonic food webs? The selective feeding of the copepods *Acartia bifilosa* and *Eurytemora affinis* was measured in order to evaluate grazing on *Dinophysis* spp. in different algae communities. The experiments were not initially designed to produce estimates of toxin transfer, although toxins were measured from some of the copepods after the experiments. *Eurytemora affinis* and *Acartia bifilosa* were chosen as grazers because they are the most common copepod species in the coastal areas of the northern Baltic, and feeding-related studies with these species have previously been performed in the study area (Kivi *et al.* 1996, Uitto 1997, Koski *et al.* 1999a, Koski *et al.* 1999b, Engström *et al.* 2000, Sopanen *et al.* 2006).

Material and methods

Ten grazing experiments were carried out during the summers of 2004 (experiments I–VI) and 2005 (experiments VII–X). The incubation water and copepods for the experiments were collected from Tvärminne Storfjärd off the SW coast of Finland, in the vicinity of the Tvärminne Zoological Station (western Gulf of Finland) (59°49'N, 23°17'E). For a detailed description of the study area *see* Niemi (1975).

Incubation water, copepods and experimental setup

The incubation water was collected below the thermocline (situated at 11–15 meters) and filtered first through a 76 µm net to remove zoo-

plankton and larger cells, such as filamentous cyanobacteria. Since the natural *Dinophysis* spp. concentrations were low for experimental purposes (typically 1–5 cells ml⁻¹), the water was concentrated by inverse filtration through a 20 μ m net. For experiments I–VI, the inverse filtration was done once. Because we wanted to reduce the relative number of small phytoplankton cells as compared with *Dinophysis* spp. in experiments VII–X, we re-diluted the 20–76 μ m fraction with GF/F-filtered (Whatman) seawater, and repeated the inverse filtration 3–4 times. The resulting water was stored for 12 h under dim light in a 2-l polycarbonate bottle that was partly covered with aluminum foil and lighted from above (dim light) to attract the actively swimming cells, including *Dinophysis* spp. Thereafter, the upper part of the water was gently aspirated and used in the experiments. The initial numbers of *Dinophysis* spp. in the experimental water were adjusted to concentrations higher than those usually observed in non-bloom conditions to increase the encounter rates between copepods and dinoflagellates (see Tables 1 and 2). The initial algae communities differed between the years which affected the grazing parameters. Here we discuss the results in two parts: experiments I–VI (2004) and experiments VII–X (2005).

Copepods were collected with a 100- μ m closable plankton net from approximately 25-m depth to the thermocline and transferred to a temperature-controlled room at *in situ* temperature (10–12 °C). Adult females were hand-picked from the net material to GF/F filtered seawater and acclimated to experimental conditions for 24 h.

Adult females of *E. affinis* were incubated in 130 ml transparent glass bottles in experiments I–VI. The number of copepods in the bottles varied from 3 to 10. For each experiment, four replicate units with copepods and three controls were prepared. The bottles were filled completely, avoiding air bubbles, and placed in a plankton wheel in a thermostated water bath (rotating at 1 rpm., temperature +11 °C, 12:12 h L:D cycle, dim light). In experiments VII–X (VII and IX *E. affinis*; VIII and X *A. biflosa*) we increased the number of replicates, and incubated one copepod per 30-ml unit. For both

copepod species, 15 replicates and three grazing controls were prepared. After 24-h incubations the content of each bottle was poured through a 200- μ m sieve and copepods were transferred to petri dishes with a small volume of filtered seawater, counted and their condition was checked. Copepods from experiments I–VI were used for toxin measurements (see Toxin sample preparation and determination of DSP toxins by LC-MS). The incubation water from each replicate and control was preserved with 1% (final conc.) acid Lugol's solution (Hällfors *et al.* 1979) for plankton counts.

Cell counts and carbon conversions

Phytoplankton and microprotozoans in each grazing experiment were counted from duplicate 10 or 25 ml settled samples (Utermöhl 1958) under an inverted microscope (Leica DMIL, 100–400 \times magnifications). Cell volumes were estimated on the basis of different geometric forms (Edler 1979, Olenina *et al.* 2006) and converted to carbon. A carbon-to-volume conversion factor of 0.19 (Putt and Stoecker 1989) was used for ciliates; for dinoflagellates and taxonomically diverse protist plankton the volume-based conversions (Menden-Deuer and Lessard 2000) were used, and for unidentified autotrophs a carbon-to-volume factor of 0.11 was used (Edler 1979).

Grazing parameters and selection

Grazing (clearance and ingestion rates) on any particular food was calculated according to Frost (1972), based on the decrease in numbers of food cells in bottles containing copepods as compared with their numbers in controls without copepods. The weight-specific ingestion rates were calculated assuming that the carbon content of each individual copepod was 3.6 μ g for *E. affinis* and 1.6 μ g for *A. biflosa* (Koski 1999). For further estimates of selectivity, species were combined as food groups based on their size and category (see Table 1). The selection coefficient α was used to evaluate prey selection (Chesson 1978). α was estimated as the ratio of the clearance

Table 1. The initial proportion of different species/species groups and the total microplankton cell numbers (cells ml⁻¹) and biomass (µg C ml⁻¹) in each experiment. Prey groups were formed according to their size as follows: Experiments I–VI; Cil > 30 µm = larger ciliates including e.g. *Euplates* sp., *Tintinnopsis beroidea*, *Strobilidium spiralis*; Cil < 30 µm = smaller ciliates; *Strombidium* sp., *Mesodinium pulex*, *Myrionecta rubra*; Auto = phototrophic dinoflagellates including *Heterocapsa rotundatum*, *Gymnodinium vestificii*, *Gymnodinium* spp. + other autotrophs. Experiments VII–X; Cil_{tot} = all ciliates including *M. rubra*, Auto = phototrophic dinoflagellates + other autotrophs.

Experiment	I and II		III and IV		V and VI		VII		VIII		IX and X	
	Cells (%)	Carbon (%)	Cells (%)	Carbon (%)	Cells (%)	Carbon (%)	Cells (%)	Carbon (%)	Cells (%)	Carbon (%)	Cells (%)	Carbon (%)
<i>Dinophysis</i> spp.	11.5	38.4	6.1	29.2	21.7	53.3	57.3	81.4	64.2	84.1	30.4	65.9
<i>H. triquetra</i>	74.2	35.9	88.5	61.2	55.3	19.8	27.2	5.6	19.2	3.7	58.4	18.5
Cil > 30 µm	6.2	19.3	0.9	4.7	7.2	12.5						
Cil < 30 µm	6.7	4.0	3.3	2.2	6.5	3.3						
Cil _{tot}							3.4	4.7	3.9	4.2	2.2	6.2
Auto	1.4	2.2	1.2	2.7	9.3	11.4	12.1	8.3	12.7	8.0	9.0	9.4
Total (cells ml ⁻¹)	375		1261		273		81		63		141	
Total (µg C ml ⁻¹)		0.23		0.6		0.2		0.1		0.1		0.2

Table 2. Initial prey cell concentrations and copepod ingestion rates of *Heterocapsa triquetra* and *Dinophysis* spp. (mean ± SE) in each experiment (*n* = number of copepods in experimental units).

Species	<i>N</i>	Exp.	Initial prey concentration (cells ml ⁻¹)			Total	Ingestion rate (cells ind ⁻¹ day ⁻¹)		Ingestion rate (µg C ind ⁻¹ day ⁻¹)	
			<i>Dinophysis</i>	<i>H. triquetra</i>			<i>Dinophysis</i>	<i>H. triquetra</i>	<i>Dinophysis</i>	<i>H. triquetra</i>
<i>E. affinis</i>	3	I	43	278		374	85 ± 46	1271 ± 1674	0.19 ± 0.10	0.41 ± 0.54
	6	II	43	278		374	226 ± 71	4127 ± 1441	0.50 ± 0.16	1.33 ± 0.46
	6	III	77	1116		1262	-118 ± 95	5610 ± 577	-0.20 ± 0.21	1.81 ± 0.19
	10	IV	77	1116		1262	-1 ± 41	6073 ± 783	-0.001 ± 0.09	1.95 ± 0.25
	6	V	59	151		275	-124 ± 24	2465 ± 251	-0.28 ± 0.05	0.79 ± 0.08
	10	VI	59	151		275	40 ± 15	1766 ± 133	0.09 ± 0.03	0.57 ± 0.04
<i>E. affinis</i>	1	VII	47	22		81	1 ± 0.23	307 ± 82	0.002 ± 0.08	0.11 ± 0.08
<i>A. bifilosa</i>	1	VIII	40	12		63	43 ± 59	66 ± 31	0.10 ± 0.13	0.02 ± 0.13
<i>E. affinis</i>	1	IX	43	83		141	80 ± 29	810 ± 134	0.18 ± 0.06	0.26 ± 0.04
<i>A. bifilosa</i>	1	X	43	83		141	64 ± 41	288 ± 153	0.14 ± 0.09	0.1 ± 0.05

rates for one prey group to the sum of clearance rates for all prey groups. α varies between 0 and 1. Particular food group is neither selected nor avoided (i.e. this food group is grazed proportionally to its availability) if $\alpha = m^{-1}$ (m = number of food groups available): for experiments I–VI this is when $\alpha = 0.2$ (number of food groups = 5) and for experiments VII–X when $\alpha = 0.25$ (number of food groups = 4). When $\alpha > m^{-1}$ the food group is selected, and when $\alpha < m^{-1}$, the selection is negative.

Toxin sample preparation and determination of DSP toxins by LC-MS

The copepod toxin content was determined at the end of experiments I–VI. We combined animals from all the replicates in experiments I–VI into three pooled samples (I and II, III and IV, V and VI) to get enough material for the analyses. The copepods were washed thoroughly with GF/F-filtered seawater on glass fibre filters (Whatman GF/F), and immediately frozen. Filters with sample material were homogenized with 1 ml methanol/water (80/20) using an ultrasonic probe. The suspension was centrifuged and filtered.

The LC-MS/MS determination of the different DSP toxins was achieved by application of a modified method developed by McNabb *et al.* (2005). Chromatographic separation was carried out using a Phenomenex Luna C18(2) column 150 mm \times 2.0 mm i.d. 5 μ m (Phenomenex, Aschaffenburg, Germany) and with gradient elution from 10% to 90% acetonitrile in water containing a constant concentration of 53 mM formic acid and 5 mM ammonia formate to provide buffering. The flow rate was 300 μ l min⁻¹, and the injection volume was 20 μ l for both sample extracts and calibration solutions.

The HPLC system was equipped with a PE series 200 quaternary pump and a PE series 200 auto sampler (Perkin Elmer, Ueberlingen, Germany). All measurements were performed using an API 165 SCIEX mass spectrometer with a pneumatic-assisted atmospheric pressure ion (API) source (AB Applied Biosystems, Darmstadt, Germany). The electrospray ionisation interface operated in positive mode. Selected

ion monitoring (SIM) was performed using the following mass-to-charge (m/z) ratios: $[M + H]^+$, $[M + NH_4]^+$, $[M - H_2O + H]^+$, and $[M + Na]^+$ for OA, DTXs and $[M + NH_4]^+$ and $[M + Na]^+$ for PTXs. PTX2 was quantified based on the ammonia adduct by comparison with a certified reference solution of PTX2, obtained from the NRC, Canada. PTX2 SA was quantified by reference to PTX2 and its concentration is expressed as equivalents of PTX2.

Statistics

Differences in the weight-specific ingestion rates between different prey groups were tested with a 1-way ANOVA. If conditions for the ANOVA were not met (normality and equality of variances), we used a non-parametric Kruskal-Wallis test. All pairwise comparisons were made with a Tukey post hoc test. A non-parametric Mann-Whitney rank sum test was used to assess whether the selection coefficients for the different prey groups were significantly different from the non-selection value ($\alpha = m^{-1}$). Linear regression was calculated to examine the dependence of clearance and ingestion rates of the copepods on initial prey cell concentration in different experiments. The analyses were performed with Sigma Stat for Windows 3.0.1. (SPSS) software.

Results

Prey community structure

The experimental food communities differed between years. In the first set of experiments (I–VI) the total microplankton cell concentrations (Table 1) were higher (275–1262 cells ml⁻¹) than in experiments VII–X (63–141 cells ml⁻¹). The dinoflagellate *Heterocapsa triquetra* dominated, whereas the share of *Dinophysis* spp. cells was lower. In the second set of experiments (VII–X), the proportions of *H. triquetra* and *Dinophysis* spp. cells were more equal. In all experiments, the proportion of ciliates and other dinoflagellates as well as other microprotists and autotrophs was of small importance.

Grazing

Experiments I–VI

The mean initial proportion of copepod carbon to total prey carbon was 67%. The total clearance and ingestion rates of *Eurytemora affinis* varied between 2.3–7.7 ml ind⁻¹ h⁻¹ and 1780–6530 cells ind⁻¹ d⁻¹ (0.87–2.46 µg C ind⁻¹ d⁻¹), respectively. The total ingestion rates corresponded to 25%–65% of the copepod body carbon d⁻¹. The ingestion rates for *Dinophysis* spp. ranged from negative to 226 cells (0.50 µg C) ind⁻¹ day⁻¹. The dominant algae species, *H. triquetra*, formed the main part of the diet of *E. affinis* (Table 2 and Fig. 1a). The weight-specific ingestion rates for *H. triquetra* by *E. affinis* were significantly higher than ingestion rates for other prey species in experiments II–V (Fig. 1a). Other prey groups formed a minor part of the *E. affinis* diet and were eaten in proportion to their initial concentrations. In experiments I, II and VI, *Dinophysis* spp. were grazed upon to some extent (Fig. 2). In experiments III, IV and V, the mean weight-specific ingestion for *Dinophysis* spp. was close to zero or negative (Fig. 1a).

Experiments VII–X

The mean initial proportion of copepod carbon to total food carbon was 90% for *E. affinis* and 40% for *A. biflosa*. The change in initial food concentration in different experiments in 2004 and 2005 had no apparent effect on clearance in this study ($r^2 = 0.01$, $p = 0.78$). The ingestion rates of the copepods increased with increasing food concentration ($r^2 = 0.62$, $p = 0.007$). The total clearance and ingestion rates of *E. affinis* were 2.4–4.8 ml ind⁻¹ h⁻¹ and 466–870 cells ind⁻¹ d⁻¹ (0.3–0.54 µg C ind⁻¹ d⁻¹), respectively, corresponding to 8.3%–15% of the copepod body carbon d⁻¹. The ingestion rates for *Dinophysis* spp. by *E. affinis* were 1–80 cells (0.002–0.18 µg C) ind⁻¹ d⁻¹. For *Acartia biflosa*, the total clearance and ingestion rates were 1.1–2.0 ml ind⁻¹ h⁻¹ and 138–261 cells ind⁻¹ d⁻¹ (0.13 and 0.24 µg C ind⁻¹ d⁻¹), equaling 8.1%–31.2% of copepod body carbon d⁻¹. *Dinophysis* spp. were ingested at rates of 43–64 cells (0.1–0.14 µg C) ind⁻¹ d⁻¹. Compared

with experiments I–VI, weight-specific ingestion rates for *H. triquetra* in experiments VII–X were lower, whereas ingestion rate for *Dinophysis* spp. was in the same range. As opposed to experiments I–VI, no negative grazing on of *Dinophysis* sp. was recorded in experiments VIII–X (Table 2 and Fig. 1b), whereas in experiment VII grazing on *Dinophysis* spp. was close to zero. The weight-specific ingestion rates for *Dinophysis* spp. were higher than those of *H. triquetra* in experiments VIII and X (Fig. 1b).

Food selection

The selection coefficients of *E. affinis* in experiments I–VI for *Dinophysis* spp. were lower than the non-selection value α in all experiments, whereas all other prey groups were positively selected. However, the coefficients did not differ significantly from the non-selection α value (Table 3 and Fig. 3). In experiments VII–X, the selection patterns of *E. affinis* and *A. biflosa* for different prey groups were similar. Neither of the copepod species selected *Dinophysis* spp., while ciliates and the dinoflagellate *H. triquetra* were selected (Table 3).

Survival and toxin retention

Ingestion of *Dinophysis* spp. did not have any negative effects on copepods. The copepods were actively swimming at the end of the experiments and the survival (mean \pm SE) remained high: 91% \pm 3% for *E. affinis* and 100% for *A. biflosa*. PTX2 was detected in the copepods from two of the three pooled samples from 2004. These concentrations correspond to 142 and 53 pg PTX2 (exp. I and II, and III and IV, respectively) per one *E. affinis* individual.

Discussion

Feeding rates

Studies of copepod grazing on *Dinophysis* spp. have given variable results. The oceanic calanoid *Calanus helgolandicus* was found to feed on *D. norvegica* efficiently at ingestion rates of

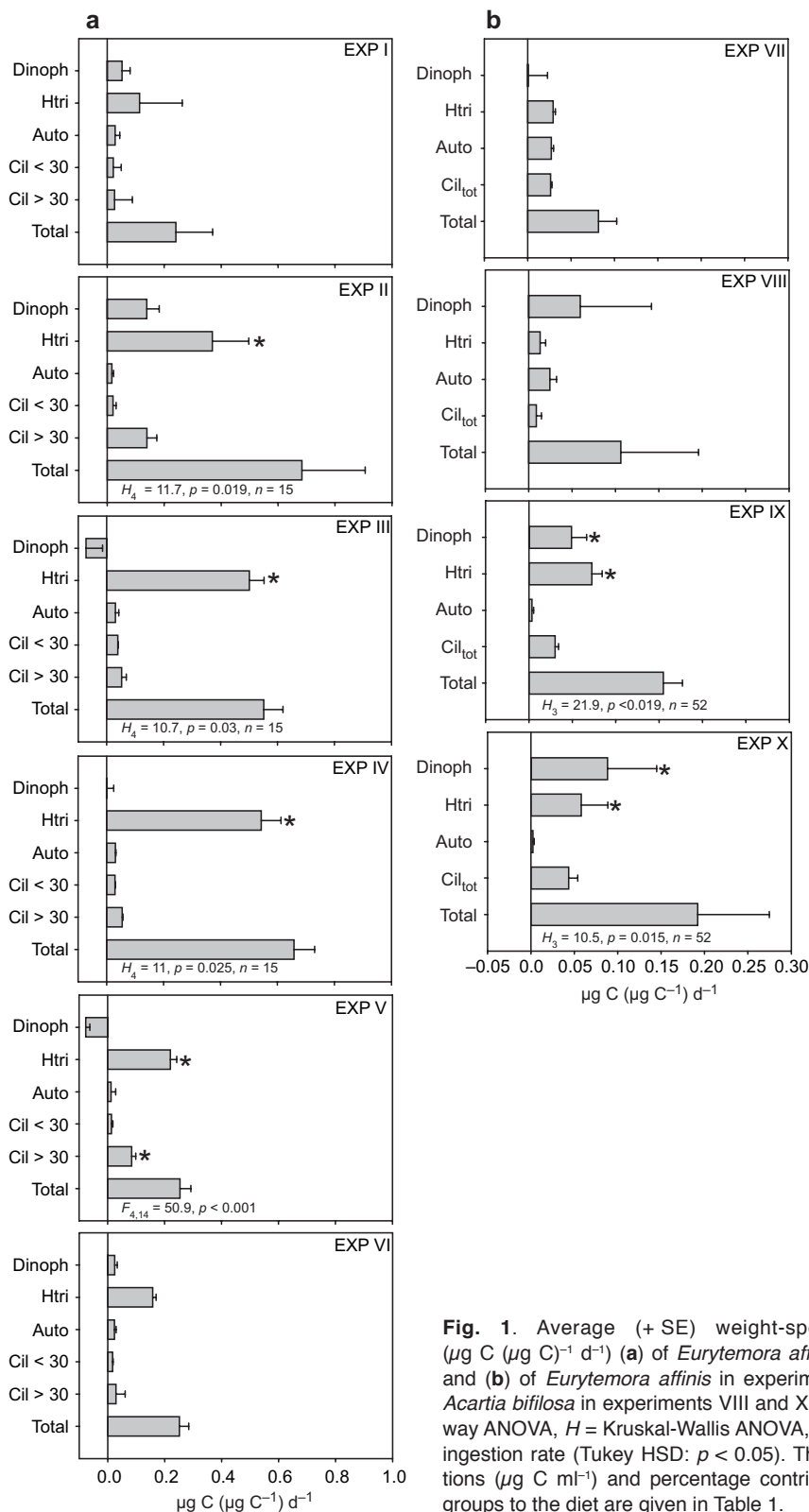


Fig. 1. Average (+ SE) weight-specific ingestion rates ($\mu\text{g C } (\mu\text{g C}^{-1}) \text{ d}^{-1}$) (a) of *Eurytemora affinis* in experiments I–VI; and (b) of *Eurytemora affinis* in experiments VII and IX, and of *Acartia bifilosa* in experiments VIII and X. Abbreviations: F = one-way ANOVA, H = Kruskal-Wallis ANOVA, * = significantly different ingestion rate (Tukey HSD: $p < 0.05$). The initial food concentrations ($\mu\text{g C ml}^{-1}$) and percentage contributions of different food groups to the diet are given in Table 1.

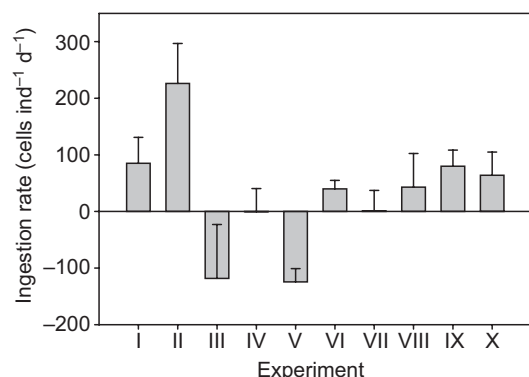


Fig. 2. Ingestion rates (mean \pm SE) for *Dinophysis* spp. in the experiments.

up to 1128 cells ind⁻¹ day⁻¹ (Jansen *et al.* 2006), whereas *Acartia* spp. both avoided (Maneiro *et al.* 2000, Jansen *et al.* 2006) as well as selected (Carlsson *et al.* 1995, Kozłowsky-Suzuki *et al.* 2006) *Dinophysis* spp. Our results are in agreement with those obtained by Kozłowsky-Suzuki *et al.* (2006), who carried out experiments with concentrated plankton communities from the northern and southern parts of the Baltic Sea. They found that the mean ingestion rates of the copepod *A. biflosa* feeding on *Dinophysis norvegica* varied from 12 to 150 ng C ind⁻¹ h⁻¹ at different algae concentrations. In these experiments *D. norvegica* dominated, constituting 90% of the carbon in each algae community. The authors concluded that increases in ingestion

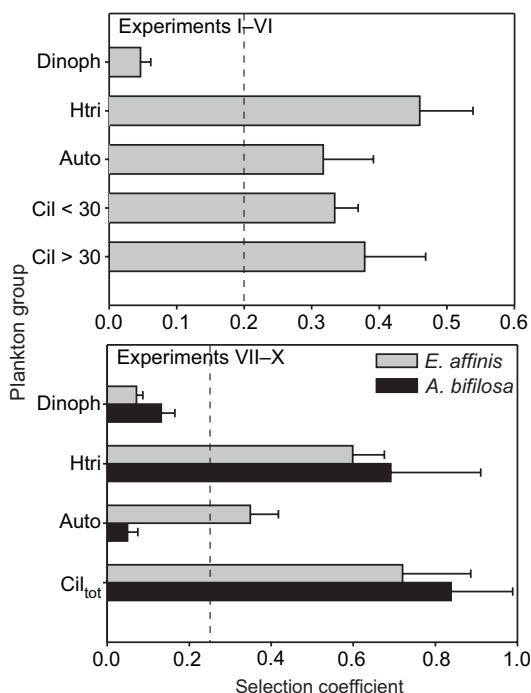


Fig. 3. Pooled (mean \pm SE) selection coefficients (α) for *Eurytemora affinis* (grey bars) and *Acartia biflosa* (black bars). Dashed lines indicate $\alpha = m^{-1}$ (i.e. neither selection nor avoidance).

rates (total and *Dinophysis* spp. ingestion) were due to the increase in food availability.

Our results for copepod ingestion of *Dinophysis* spp. (< 0 up to $0.5 \mu\text{g ind}^{-1} \text{ day}^{-1}$) are at the

Table 3. Selection coefficients (α) (mean \pm SE) for *Eurytemora affinis* (exp. I–VII, IX) and *Acartia biflosa* (exp. VIII, X) grazing on different algae species or groups. No selection for or against a species or group occurred when $\alpha = 0.2$ (year 2004) and $\alpha = 0.25$ (year 2005). Asterisks indicate values which are significantly ($* = p < 0.05$, $** = p < 0.001$) different from 0.2 or 0.25 (Mann-Whitney Rank Sum test). Dinoph (*Dinophysis* spp.); Htri (*Heterocapsa triquetra*); Cil $< 30 \mu\text{m}$, Cil $> 30 \mu\text{m}$, Cil_{tot} (all ciliates including *Myrionecta rubra*); Auto (dinoflagellates and other autotrophs).

Experiment	Dinoph	Htri	Cil $< 30 \mu\text{m}$	Cil $> 30 \mu\text{m}$	Cil _{tot}	Auto
2004						
I	0.09 ± 0.06	0.2 ± 0.2	0.8 ± 0.4	0.2 ± 0.05	–	0.6 ± 0.3
II	0.1 ± 0.01	0.4 ± 0.03	0.2 ± 0.07	0.3 ± 0.09	–	0.3 ± 0.02
III	0.006 ± 0.006	0.2 ± 0.001	0.3 ± 0.1	0.5 ± 0.09	–	0.4 ± 0.2
IV	0.01 ± 0.01	0.3 ± 0.02	0.4 ± 0.01	0.3 ± 0.02	–	0.4 ± 0.01
V	0.0 ± 0.0	0.9 ± 0.2	0.2 ± 0.06	0.5 ± 0.05	–	0.05 ± 0.05
VI	0.02 ± 0.009	0.7 ± 0.2	0.3 ± 0.05	0.3 ± 0.08	–	0.1 ± 0.02
2005						
VII	$0.04 \pm 0.02^{**}$	$0.6 \pm 0.1^{*}$	–	–	$0.5 \pm 0.3^{**}$	0.6 ± 0.07
VIII	$0.1 \pm 0.06^{*}$	$1.0 \pm 0.6^{*}$	–	–	$0.6 \pm 0.2^{*}$	0.2 ± 0.07
IX	$0.1 \pm 0.02^{**}$	0.6 ± 0.1	–	–	$0.9 \pm 0.2^{*}$	$0.03 \pm 0.01^{**}$
X	$0.1 \pm 0.04^{*}$	0.5 ± 0.1	–	–	0.9 ± 0.3	$0.0 \pm 0.0^{**}$

lower end of what was reported by Kozlowsky-Suzuki *et al.* (2006). They also studied grazing of the copepods *Temora longicornis* and *Centropages typicus* in the southern Baltic on plankton communities containing small amounts of *Dinophysis acuta* (ca. 3%–21% of the total food carbon). Ingestion rates for *D. acuta* in these experiments were in the same range as in our study, ranging between 5 and 40 ng ind⁻¹ h⁻¹.

Feeding parameters for the two copepod species in our study can be directly compared between experiments IX and X, which were carried out with the same algae community. There, the overall ingestion rates of *A. bifilosa* were less than 50% of the ingestion rates of *E. affinis*, but feeding on *Dinophysis* spp. contributed a substantial part of the weight-specific ingestion of *A. bifilosa*. These results may be related to their different feeding modes, but with only a few observations no conclusions can be made.

Prey selection

According to the estimates of Kozlowsky-Suzuki *et al.* (2006), copepods did not select for *Dinophysis* spp. although they were actively feeding on them, and the authors concluded that with a high grazer concentration and poor growth conditions for *Dinophysis* spp. the grazers are able to control the growth of the dinoflagellates. The grazing of the copepods *C. typicus* and *T. longicornis* caused significant negative effects on *Dinophysis* spp. growth at low food concentrations, whereas the effects of *A. bifilosa* grazing on the dinoflagellates were significant at higher food concentrations.

Our findings on prey selection agree well with these results. Our first experiments (I–VI) indicated that *Dinophysis* spp. were not preferred food for the copepods, but because of their relatively large cell volume (Olenina *et al.* 2006) the observed ingestion rates resulted in reasonably high weight-specific ingestion in some of the experiments. When the toxin content of the copepods used in these experiments was analyzed, PTX2 and PTX2SA were detected. We wanted to study further the interactions of the common copepods and *Dinophysis* spp. and especially test, whether low preferences for *Dinophysis* spp.

by *E. affinis* were connected to the dominance of *Heterocapsa triquetra* in the incubation water. In experiments VII–X, the relative proportions of *Dinophysis* spp. were adjusted to be close to the concentrations of *H. triquetra*, which also diluted the total algae concentration, leading to lower total ingestion rates. In these experiments, the relative importance of *Dinophysis* carbon in the copepod diet was higher, which was mainly due to low total cell concentration and the relatively large size of the cells, not increased grazing or selection. Although the total food carbon in experiments VII–X was markedly lower than in the first set of experiments, and the share of *Dinophysis* spp. higher, no positive selection for *Dinophysis* spp. was found.

The dinoflagellate *Heterocapsa triquetra* was the most preferred algae species in all our experiments. Several explanations for the high ingestion rates for *H. triquetra* as compared with those for *Dinophysis* spp. are possible, and on the basis of our results we cannot state whether the selection we observed was: (1) for a better food type, or (2) against a non-preferred algae. Calanoid copepods, in general, are selective feeders that display flexibility in their feeding behavior (Koehl and Stickler 1981). Discrimination between food types can be based e.g. on size (Frost 1972), motility (Atkinson 1995) and chemical signals (Cowles *et al.* 1988, Colin and Dam 2003). *Eurytemora affinis* is a suspension feeder and considered less selective than species of the genus *Acartia* that are able to switch between filtering-type and the more active ambush-and-attack type of foraging (Tiselius and Jonsson 1990, Kiorboe *et al.* 1996). Both these copepod species are relatively small (adults 1.0–1.5 mm) as compared with oceanic species like *Calanus* spp. that can be from 2.5 to 10 mm in length.

Copepods are able to feed on cells ranging in size from approximately 5 to 200 μ m (Gasparini and Castel 1997) depending on the size of the copepod (Hansen *et al.* 1994), and retain the largest particles more efficiently than the smallest ones (Frost 1972). However, in coastal waters copepods often prefer the usually abundant nanoplankton (Kivi *et al.* 1996, Gasparini and Castel 1997, Koski *et al.* 1999b). During late summer in the northern Baltic Sea, when *Dinophysis* spp.

are most abundant, the plankton communities are characterized by filamentous and colonial nitrogen-fixing cyanobacteria, various cryptophytes, chlorophytes, chrysophytes and dinoflagellates (Kononen 1988, Kononen *et al.* 2003, Suikkanen *et al.* 2007). Besides filamentous cyanobacteria the majority of these are relatively small nanoplanktonic species in the food size range of the copepods. Suitable sized or good quality food, like *Heterocapsa triquetra* (Uye and Takamatsu 1990) that regularly form blooms during late summer, may be more preferred and thus lower the grazing pressure on *Dinophysis*, which may also be neglected because of their relatively large size (Jansen *et al.* 2006). Copepods may also avoid *Dinophysis* spp. because of their toxicity, but to our knowledge no studies have shown clear harmful effects of DSP toxins on zooplankton. OA and DTXs have been suggested to have allelopathic effects on the growth of microalgae (Windust *et al.* 1996), but to our knowledge no such information for pectenotoxins exists for any planktonic organisms. In the study of Carlsson *et al.* (1995), *Acartia clausi* actively selected *Dinophysis acuminata* and experienced elevated mortality during the grazing experiment. The toxicity of the *Dinophysis* cells in that study was not analysed, and in any case the suspected toxicity did not prevent the copepods from feeding on *D. acuminata*. We cannot rule out toxic effects, because we did not measure algal toxin content, nor toxin transfer from algae to copepods in our study.

Toxin content

We found PTX2 in the animals after incubation in two combined samples (exp. I and II: 142 pg ind⁻¹, III and IV: 53 pg ind⁻¹). To our knowledge these are the first observations of pectenotoxins in copepods. It is worth noting that our calculated mean ingestion rates in experiments III and IV were negative for *Dinophysis* spp. However, the results from these experiments included also positive ingestion estimates (28 to 78 cells ind⁻¹ day⁻¹) that may well have been responsible for the presence of the toxins in copepods, since the measured toxin concentrations in the copepods corresponded to the

toxin content of < 100 cells, depending on the dinoflagellate cell toxin content. These approximations are based on the reports of Kuuppo *et al.* (2005), who found PTX2 concentrations in *Dinophysis* spp. of the study area varying between 1.6 and 19.2 pg cell⁻¹.

PTX2 is usually detected only in phytoplankton assemblages, and not in shellfish, which has been explained by the rapid oxidation of PTX2 in shellfish tissues (Lee *et al.* 1988, Draisci *et al.* 1996). In the recent study of Blanco *et al.* (2007) PTX2 as well as PTX2SA were detected in filter feeders like shellfish. Curiously, in their study, PTXs were detected both in plankton and shellfish, but no trace of OA was found. Feeding on *Dinophysis* spp. cells does not necessarily indicate digestion or toxin accumulation in the grazer. In the study of Wexels Riser *et al.* (2003), the ingested *Dinophysis* cells were found intact in the faecal pellets of *Calanus helgolandicus*, and similar observations have also been reported for *Temora longicornis* (Maneiro *et al.* 2000, Maneiro *et al.* 2002). Our detection of PTX2 in copepods after the incubations suggests that toxins were not instantaneously metabolized in zooplankton as they usually are in shellfish. To get more information on this, the toxin content of the faecal pellets should also be measured, and the pellet contents studied microscopically. Since we did not study the faecal pellets, we do not know whether the *Dinophysis* cells were actually digested, or just passed through the copepods. Whether the copepods accumulate the toxins or not, they may nevertheless be toxic after ingestion of toxin-containing cells, and thus be vectors themselves, or produce toxic faecal pellets.

Kuuppo *et al.* (2006) measured *Dinophysis*-derived toxins from phytoplankton communities and settling organic material on the SW coast of Finland. Their sediment trap material contained PTX2 and DTX1, but no *Dinophysis* spp. cells. The trap material, however, included copepod faecal pellets that were suspected to be the sources of these toxins. They estimated that only 0.01% of the PTX2 and 0.1% of the DTX1 that was found suspended in the water column was present in the trap material. Such low toxin concentrations in pellets are in agreement with our results on the low copepod feeding rates on *Dinophysis* spp. cells. We also conducted field

studies on toxin distribution in plankton communities in the study area, and have detected pectenotoxins in phytoplankton communities but not in zooplankton (O. Setälä unpubl. data). On the basis of these observations it seems thus likely that during times when other, more preferred food sources are abundant, the copepods in the study area do not have a significant role in the toxin transfer to higher levels of the food web. However, at times when the total algae numbers are low, *Dinophysis* spp. may constitute a substantial part of the copepods' diet.

Applicability of the results

We made an effort to study the relationships between the grazers and *Dinophysis* spp. with an algae community that would be close to what is found in natural conditions, and therefore used "semi-natural" i.e. field communities that were manipulated to adjust the relative abundances of different algae species. Grazing experiments with zooplankton and their prey are often made with cultured algae, and with very high cell concentrations, which can make it difficult to relate the results to natural environments, which we wanted to avoid. More applicable results can be obtained from studies performed with algae communities of naturally co-occurring species, in concentrations actually occurring in natural environments (Turner *et al.* 1998, Jansen *et al.* 2006).

The total initial concentrations in the experiments ranged from <100 to approx. 1300 cells ml⁻¹, which are low as compared with values given in many other copepod grazing studies, but are in the range of natural concentrations of nano- and microplankton (Kononen 1988, Setälä and Kivi 2003, Suikkanen *et al.* 2007) in the area. The *Dinophysis* spp. concentrations in the experiments (40–77 cells ml⁻¹) were higher than what is typically seen in the study area during non-bloom conditions (Kuuppo *et al.* 2006, Hajdu *et al.* 2007). We adjusted the initial concentrations to these levels in order to increase the encounter rate of the copepods with *Dinophysis* spp. cells and also to establish stability in grazing parameters. Since considerably higher *Dinophysis* spp. concentrations (up to 150 cells ml⁻¹) have been observed in subsurface

layers (Carpenter *et al.* 1995, Setälä *et al.* 2005, Hajdu *et al.* 2007), concentrations as high as in our experiments are also likely to be encountered by these vertically migrating copepods (Burris 1980).

Conclusions

Our experiments were run with microplankton communities where both the total cell numbers as well as the proportion of *Dinophysis* spp. were realistic for the study area in late summer. Several experiments were carried out with incubation water that was dominated by the dinoflagellate *Heterocapsa triquetra*, a species which often predominates in late summer phytoplankton communities in the study area (Kononen *et al.* 2003). Our finding of non-selective feeding on *Dinophysis* spp. in the presence of high numbers of *H. triquetra* reflects selection for this species as food. In experiments with more dilute algae communities, where the share of *Dinophysis* spp. was relatively high, the overall ingestion rates were lower and *Dinophysis* spp. were not selected. This suggests that *Dinophysis* spp., although included in their diet, are not preferred by the two common copepod species in the area, but at times of low food availability they may be an important food item for the grazers. Since we did detect pectenotoxins in the copepods, these copepods may have at least a minor role in the transfer of toxins to higher levels in planktonic food webs as well as to benthic animals.

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